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Treatments of honey bee colonies affected by *Varroa destructor* in organic apiculture

A. Nanetti, A. Rovida, P. Piazza, A. Martini, A.M. Besana and M.P. Tampieri

***Abstract* – The organic honey bee productions are provided for in the EU Regulation 1804/99. Notwithstanding the beekeeping industry deserves a considerable economic importance to the whole organic market of the EU, limited action is paid in favour of the organic beekeeping. A noticeable exception to this are the huge research efforts on the disease control, which is mainly due to the lack of useful and not contaminating remedies against severe pests hitting the honey bees in the whole EU.**
To avoid the honey contamination with treatment residues is paramount and fosters researche on soft chemicals like oxalic acid, which is of utmost importance not only in organic, but also in conventional contexts.
The Authors are presenting here a synthetic review on the treatments against the varroosis with OA and on their research to optimise the use of the substance in different Italian environments.[1]

A GENERAL INTRODUCTION

Organic honey bee productions are an important part of the EU Regulation CE 1804/99, and the organic honey production have a big chance today, due to the concern about safety of conventional active ingredients and to residues that possibly accumulate in the products. Although this sector is interesting to the organic EU market, limited research efforts have been done, apart from the control of the major bee pests. A big attention is focused on the residue issue and on disease management techniques avoiding the honey bee products (honey, wax etc.) being jeopardised by residues. The oxalic acid (OA) is one of the best solutions for the organic treatment of the external parasite *Varroa destructor*, which is a paramount problem both in organic and conventional beekeeping industry world-wide. With this article the Authors review the problem and summarise their own research carried out in Italy on the best use of OA in the struggle against the mite.

INTRODUCTION TO VARROA

The ectoparasitic mite *Varroa destructor* Anderson & Trueman (Acari, Mesostigmata, Varroidae) is one of the most concerning pests hitting the honey bees (*Apis mellifera*), the basis of the planet's beekeeping industry. The mites feed on bee and brood haemolymph and rear their own offspring in the brood cells. Pathogenesis is related to host spoliation and virus transmission.

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Contrary to the original host species (*A. cerana*), honey bees do not show sufficient tolerance to control the mite populations. Hence, the infestations grow quickly and bring the bee colony to death.
Eradication is not possible, and this fostered the present endemic spread world-wide. Today acaricide treatments are the only reliable control method, but they entail a twofold problem:

- the mite may develop resistance;
- hard chemicals are potential source of contamination for the hive products.

If pesticides are found in honey, the purity that buyers expect in the product is compromised. This is detrimental to consumers, to producers and to the whole honey market. The possible wax contamination is a further huge problem. Organic beekeeping needs it is organic also, and control bodies may require residue analyses on the wax.
Many research efforts have been spent successfully and yielded sustainable, effective, simple and economically sound treatment concepts. They consist chiefly of applications of natural active ingredients, compounds of essential oils (thymol) and organic acids, namely. Among the last ones, OA is of utmost importance and is greatly appreciated by the beekeeper's community Europe-wide. Great contribution was given by the European Group for the Integrated Varroa Control.

WHY IS OA 'NATURAL'?

Oxalates can be found naturally in many living beings, humans included, and in common foodstuffs (e.g.: spinach, rhubarb, cauliflower, peanut, eggplant, chocolate, tomato). The usual dietary oxalate intake is tens to hundreds mg/day, without expected adverse effects. Honey contains oxalates too in amounts strongly depending on the botanical type (3-760 mg/kg).
The OA is provided for in the UE Regulation 1804/99 and in the 2nd Annex to the UE Regulation 2377/90. Accordingly, organic and conventional pest control may benefit of the OA, whose concentration in the honey is not subject to any Maximum Residue Limit.

THE MODE OF ACTION OF OA

Metabolic calcium chelation is the main toxic mechanism of OA in mammals, but experiments with other chelating agents showed this is negligible in the case of varroa. Conjectures on a possible systemic action are not corroborated by investigations on pharmacodynamics that highlighted a mechanism by contact (Nanetti *et al.*, unpublished).
The OA is the simplest bicarboxylic organic acid. Lab and field experiments unveiled that the mode of action depends on the strong solution acidity, that cannot be achieved by most organic acids. This

agrees with the low efficacy exhibited by weaker acids (lactic, citric) and with trials demonstrating that the mite mortality is far more depending on concentration than on the whole OA amount given (Fries, 1999).
Consequently, the OA efficacy relies on the presence of water in the formulation, which allows the acidity to be develop in the solution, and prevents dehydration and re-crystallisation.

The treatments with OA

Short persistence within the colony makes the OA suitable for winter treatments, when all the mites are exposed to the treatment. The main available administration methods are listed below:

- Spraying: 2.1% OA solution sprinkled on the bees of each comb side;
- Trickling: syrup added with OA and dripped on the comb tops, 5 ml each, by a syringe (Nanetti & Stradi, 1997);
- Sublimation: a heater (Varrox®) loaded with OA crystals is slipped into the flight entrance and left to complete sublimation (Radetzki & Baermann 2001).

Labour and time requirements made the spraying technique obsolete. In most cases it has been replaced by the 'trickling method' and sometimes by the 'sublimation method'. The Authors compared both trickling and sublimation on broodless colonies in different environmental conditions of Italy.

Winter 2004/2005

In a cold area, the colonies of an apiary were treated by trickling with solutions at different OA/sugar concentrations or by sublimation. The recorded efficacy was slightly lower than expected on the basis of previous findings (Nanetti *et al.*, 2003). It is noteworthy that a reduced sugar concentration (30%) resulted in considerable loss of efficacy and that the treatment seemed otherwise robust to a decrease of OA concentration to 3.2% (Table 1).

Table 1. Efficacy of OA in the winter. Trickling of solutions at various concentrations and sublimation. Same letters mean non-significant difference (alpha=0.05).

Group	Treatment	N	OA	Sugar	Mites (N)	Mortality (%)
1	Trickling	10	4.2%	60%	596	87.7[a]
2	Trickling	10	4.4%	30%	510	70.3[b]
3	Trickling	10	3.2%	60%	722	87.1[a]
4	Sublimation	10	1.4 g	-	623	90.9[a]
5	Untreated	10	-	-	415	13.6

Evaporation occurs in the case of low relative humidity and discontinues the solution efficacy. Otherwise, sugar is hygroscopic and is added to keep the solution hydrated, with an effect that depends on the sugar concentration. Internal recordings showed dry conditions in the treatment area in the first week (RH: 40-70%) that the presence of sugar could not cope with.
OA sublimation was not significantly more effective, but it was complicate, time taking and unsafe for the beekeeper, who run the risk to get contaminated and to inhale OA fumes and microcrystals. Such negative points were not counterbalanced by better tolerability to the colonies. The overwintering success (number of brood cells and adult population increase) was taken into account in this trial and in a Mediterranean replicate either, but between groups differences were not significant (Nanetti *et al*. 2005).

Winter 2005/2006

In this trial, very strong colonies in a continental environment were given OA by sublimation, once or twice ten days apart, and by the trickling of a weak solution. A substantial confirmation of the sublimation efficacy was attained, but a second administration did not yield a significant increase (Table 2).
The 3.2% OA solution exhibited an unexpectedly reduced acaricidal activity, which was about 7 percent units less than the one recorded in the previous trial. In this case only, a positive dose/efficacy correlation was found, which may indicate the need of further dose adjustment when highly populated colonies are being treated.

Table 2. Efficacy of OA in the winter. One and two applications by sublimation and trickling of a weak solution. Same letters mean non-significant difference (alpha=0.05).

Group	Treatment	N	OA	Sugar	Mites (N)	Mortality (%)
1	Sublimation	9	1*1.4 g	-	486	92.2[a]
2	Sublimation	9	2*1.4 g	-	443	91.7[a]
3	Trickling	8	3.2%	60%	474	78.9[b]
5	Untreated	9	-	-	298	8.7

Conclusions

The varroa mite, the most concerning honey bee parasite world-wide, can be organically controlled with oxalic acid. Techniques for easy, safe and cheap treatments are available.
The trials showed that the *trickling of solutions* (4.2%) is highly effective in damp environments, but sugar can be added as a synergist hindering dehydration. Hence, low sugar concentrations are highly unadvisable. 3.2% solutions were active also, but wanting efficacy may be expected in strong colonies, requiring further dose adjustment.
Effective treatments may be performed by *crystal sublimation* also, but the difficult, costly and unsafe application did not seem counterbalanced by any advantage. Besides, repeated sublimations were economically unsound in the tested conditions.
The standard doses (trickling: 5 ml/comb; sublimation: 1,4 g) allowed fair tolerability to the colonies.

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